Formaldehyde Council

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Via Electronic Mail

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RE: Comments on the Recommendation from the Expert Panel Report (Part B) on Formaldehyde, 74 Fed. Reg. 67,883 (December 21, 2009)

Dear Dr. Lunn:

I. Introduction and Summary

The Formaldehyde Council, Inc. (FCI)[1] appreciates the opportunity to submit comments to the National Toxicology Program (NTP) on the recommendation and justification from the Expert Panel on the listing status of formaldehyde in the 12th Report on Carcinogens (RoC).[2] The Expert Panel, together with four non-voting technical members, met on November 2-4, 2009, to assess the scientific evidence presented in the draft Background Document (NTP 2009), and, using the RoC listing criteria, to develop a listing rationale and recommendation for formaldehyde. The Panel Report focuses on three cancer endpoints: nasopharyngeal cancer (NPC), sinonasal cancer, and myeloid leukemia. While the Panel considered the epidemiological evidence for each endpoint separately, the Panel apparently assumed that the epidemiological evidence for each of these endpoints was equally strong and therefore justified the recommended listing that formaldehyde is *known to be a human carcinogen* based on one vote for all three endpoints. These comments briefly compare the scientific data and the Panel's Report for the three identified endpoints. As with the Panel, we agree that a "weight-of-evidence" approach is the most appropriate.[3] Highlights from this comparison follow.

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[1]FCI is a group of leading formaldehyde producers and users who are dedicated to promoting the responsible use and benefits of formaldehyde and ensuring its accurate scientific evaluation. FCI members include American Forest and Paper Association; Arclin; Atlantic Methanol Company; Celanese Corporation; CertainTeed Corporation; Cytec; DB Western, Inc.; Dow Chemical Company; DSM Melamine; E. I. du Pont de Nemours and Company; Formica Corporation; GEO Specialty Chemicals; Georgia-Pacific LLC; Hexion Specialty Chemicals, Inc.; Kitchen Cabinet Manufacturers Association; Methanex Corporation; Methanol Holdings (Trinidad) Limited; National Funeral Directors Association; Owens Corning; Panolam Industries International; and Troy Corporation.

[2] Formaldehyde Expert Panel Report, Part B – Recommendation for Listing Status for Formaldehyde and Scientific Justification for the Recommendation (Dec. 16, 2009)(hereinafter referred to as "Expert Panel Report" or "the Report").

[3] Expert Panel Report at 9.

Myeloid Leukemia

The human, animal and other data are not sufficient to support a causal association between formaldehyde exposure and myeloid leukemia.

- Three of the four studies relied on by the Expert Panel (Coggon *et al.* (2003), Pinkerton *et al.* (2004) and Beane-Freeman *et al.* (2009)) state that they did not find a statistically significant association or dose-response relationship between formaldehyde exposure and myeloid leukemia.
- This consistent finding among three diverse studies on more highly exposed cohorts encompassing more than 50,000 individuals outweighs the limited results of the fourth study (Hauptmann *et al.* 2009) and demonstrates that there is not "sufficient" evidence of an association with myeloid leukemia consistent with NTP listing criteria. This is particularly evident because the largest of these studies (Beane-Freeman *et al.* 2009) unequivocally does not show a significant association between formaldehyde exposure and myeloid leukemia in a cohort of more than 25,000 exposed workers.
- Comparing observed and expected mortality in the three major human formaldehyde studies to which this method can be applied (i.e., Coggon *et al.* (2003), Pinkerton *et al.* (2004) and Beane-Freeman *et al.* (2009)), the combined observed and expected leukemia mortality data from these studies of formaldehyde exposed workers illustrate quite clearly that there is no excess of leukemia deaths.
- Meta-analyses show no consistent or collective effect regarding leukemia.
- Hauptmann (2009) evaluated deaths occurring between 1960 and 1986, including subtypes of lymphohematopoietic cancers reported on death certificates as either underlying or contributing causes of death. However, even as late as 1992, the National Cancer Institute's (NCI) Surveillance, Epidemiology and End Results (SEER) Program specifically declined to report study results on these cancers due to problems with the validity of these diagnoses as reported on death certificates. Although there was some effort to assure comparability, important differences are apparent between myeloid (acute and chronic combined) leukemia cases and the control group. The text states that alternative analyses using <500 embalmings as the referent generates more reliable results; however, the unreliable result is reported in the abstract and no real rationale is provided for using <500 embalmings as a reasonable "unexposed" cutpoint.
- Til *et al.* (1989) performed a chronic drinking water study in rats at doses of 5, 25, and 125 mg/kg. Blood samples were taken at necropsy and, after two years of formaldehyde exposure, there were no differences between dose groups in any hematological parameters, no dose-related lymphoma in axillary lymph nodes, and no evidence of myeloid leukemia in blood cells.
- Inhaled formaldehyde, even up to 15 ppm for 90 days, has no adverse effects on red or white blood cell counts or on the bone marrow of rats. M. Andersen (personal communication). These findings following 90 days exposure to 15 ppm of formaldehyde demonstrate that the initial events (i.e., myelotoxicity-driven pancytopenia) required for the development of leukemia do not occur.
- Distant-site toxicity was investigated by Lu *et al.* (in press) where rats were exposed via inhalation to ^{13}C-formaldehyde at 10 ppm for 1 and 5 days. While formaldehyde-DNA adducts from both endogenous and exogenous formaldehyde were readily detected in nasal

epithelium after 1 or 5 days, no formaldehyde-DNA adducts from exogenous formaldehyde were detected at any site distal to the nose, including blood and bone marrow. This confirms prior work demonstrating that exogenous formaldehyde does not get past the nasal epithelium, and calls into question the findings reported by Zhang *et al.* (2010) involving formaldehyde-induced changes.

- The vast majority of more credible data show essentially no reported adverse hematological effects in humans or animals following either oral or inhalation exposure to formaldehyde.

Nasopharyngeal Cancer

- The "*only cohort study that is individually informative*" for evaluating the potential carcinogenicity of formaldehyde as NPC was the NCI cohort of workers in formaldehyde industries by Hauptmann *et al.* (2004).[4]
- In that study, there was a total of 9 deaths from NPC, with 5 of the cases coming from only one plant (Plant #1) and the remaining four cases randomly occurring in the other nine plants, an atypical pattern if formaldehyde were actually the cause of NPC.
- Marsh *et al.* (2007) provides evidence that the NPC reported in the Hauptmann *et al.* (2004) cohort may not be related to formaldehyde exposure. Five of the six NPC cases at Plant #1 had previously worked in occupations involving substantial exposures to potential risk factors for upper respiratory system cancers, including sulfuric acid mists and metal dusts.
- In rat studies, nasal tumors occurred only at formaldehyde concentrations of >6 ppm. These concentrations are sufficient to cause severe cytotoxicity of the rat nasal epithelium, with subsequent regenerative proliferation observed.
- These findings are strengthened by more recent toxicogenomic studies by Andersen *et al.* (2008), which provide empirical support at the genomic level that formaldehyde exposure at concentrations of 2 ppm and less are incapable of causing tissue damage that could lead to tumor formation.

Sinonasal Cancer

- The meta-analyses that examined sinonasal cancer indicate that there is no increased risk of this cancer in the cohort studies (Bosetti *et al.* 2008, Collins *et al.* 1997).
- In Hauptmann *et al.* (2004) only three cases (nose and nasal cavity) were observed in the cohort, which were not statistically significant (SMR=1.19, 95% CI 0.38 – 3.68) (Table 2, Hauptmann *et al.* 2004).
- Based on the same data, the International Agency for Research on Cancer (IARC) concluded that there is "limited" evidence of sinonasal cancer in humans. IARC (2009).

The science does not support many of the Expert Panel Report's conclusions. This confusion may have occurred because the Panel took a single vote on the scientific evidence for all three endpoints, as opposed to assessing each endpoint individually. The data and scientific literature lead to much different conclusions.

For these reasons, we urge NTP to reconvene the Expert Panel in order that it may assess the data based on the three, separate toxicological end points using a weight-of-evidence approach

[4] Expert Panel Report at p. 4.

and then re-poll the Panel as to whether the data are "sufficient," "limited" or “inconclusive” with respect to each end point.

We note a few of the activities already underway or soon to be initiated as part of FCI and industry efforts to advance the understanding of formaldehyde toxicology and to help resolve the still substantial scientific uncertainties that remain:

- A longitudinal study of the measurement of red and white blood cell counts before, during and after formaldehyde exposures of hundreds of workers at plants where formaldehyde was either manufactured or used. The study will determine if decreased blood counts are a consequence of formaldehyde exposures. This is highly relevant and a key factor in interpreting the soundness of recent work claiming an association between formaldehyde and leukemia-related endpoints. We expect that this study will be completed by mid-2010.
- Publication of the results of the 90-day toxicogenomic study with exposures to formaldehyde up to 15 ppm (M. Andersen, manuscript in preparation). This will include the data showing that there are no adverse effects on red or white blood cell counts or on the bone marrow at any dose level.
- Requesting from the editor of the journal in which the Zhang *et al.* (2010) study was published that the authors provide additional data for review on a public website including the unpublished individual data on blood counts and archived images of fluorescence in situ hybridization (FISH) results.
- Replicating in primates the results of a recent study in rodents to confirm no delivery of exogenous formaldehyde to distant sites following exposure at 10 ppm for 1 or 5 days (or longer) to ^{13}C-formaldehyde and measured by ^{13}C-formaldehyde-DNA adducts in bone marrow or blood cells.

Based on a review of the science, the application of NTP's criteria, and regardless of whether one applies a weight-of-evidence or strength-of-evidence approach:

- the human, animal and other data for myeloid leukemia do not provide sufficient evidence that supports listing formaldehyde as either *known to be a human carcinogen or reasonably anticipated known to be a human carcinogen.*
- the human, animal and other data for nasopharyngeal cancer (NPC) do not provide sufficient evidence that supports listing formaldehyde as a *known to be a human carcinogen*, but may be interpreted to support a listing as *reasonably anticipated to be a human carcinogen*,
- the human, animal and other data for sinonasal cancer do not provide sufficient evidence that supports listing formaldehyde as either *known to be a human carcinogen* or *reasonably anticipated known to be a human carcinogen.*

This week, FCI became aware that NTP placed a final Background Document, dated January 22, 2010, on its website. In these comments, references and abbreviations are the same as those in the final Background Document and any additional references are separately listed at the end of the document. While we are using the final Background Document for citation purposes, we stress that FCI has not had the opportunity to compare the draft and final Background Document and these comments are not intended to endorse or disagree with the substance of the final Background Document.

II. Myeloid Leukemia

A. *Human Data on Myeloid Leukemia*

According to the Panel, four studies played a "key role" in the evaluation of the association between formaldehyde and leukemia, and based on these four studies, the Panel concluded that the *"strongest evidence for an association between formaldehyde exposure and leukemia is for myeloid leukemia*."[5] The four studies referenced are Coggon *et al.* (2003), Pinkerton *et al.* (2004), Beane Freeman *et al.* (2009), and Hauptmann *et al.* (2009).

FCI agrees that these are the leading human studies related to formaldehyde and worker exposure. Table 1 compares the studies' conclusions and the Panel's characterization.

Table 1. Comparison of Key Study Findings and Expert Panel Statements

Study	Study Conclusions	Panel Statements
Coggon *et al.* 2003	No significant increase in all forms of leukemia. Note: this study likely involved the highest exposures to formaldehyde of the four key studies relied upon.	"*SMRs were calculated contrasting leukemia rates for all workers with the rates for the referent population. No excess of leukemia was observed in the overall cohort, or in the smaller subgroup of men judged ever to have had high exposure to formaldehyde. Analyses of myeloid leukemia were not reported separately*."[6]
Pinkerton *et al.* 2004	Considered all leukemias; trend data were not reported. For myeloid leukemia, there was no statistical significance. While a statistically significant SMR after more than 20 years of exposure was reported in Table 4, the CIs in the text show that neither of the SMRs for 10 or 20 years of exposure were significant; a significant trend was not detected. In addition, a "multiple cause of death" (MCOD) analysis, which is not standard practice, was also conducted. Such analyses include other conditions that may be noted on death certificates but were not the cause of death for that individual. Using this procedure, there was no significant increase in myeloid leukemia in the entire cohort	"*An excess of myeloid leukemia was reported in this cohort*."[7] [Note that there was *no statistically significant* excess.]

[5] Expert Panel Report at pp. 7-8.

[6] Expert Panel Report at p. 9.

[7] Expert Panel Report at p. 8.

Study	Study Conclusions	Panel Statements
Beane Freeman *et al.* 2009	While all leukemia was significantly elevated for the peak exposure metric, myeloid leukemia was not significantly elevated based on peak exposure and the trend was also not significant (P_{trend} =.13 and .07 compared to exposed and unexposed workers, respectively). See, Table 2. "*No statistically significant associations were observed with average intensity (Table 3) or cumulative exposure to formaldehyde (Table 4)*."[8]	"*Beane Freeman et al. (2009) found elevated relative risks for leukemia, in particular for myeloid leukemia, when contrasting the highest to lowest groups defined on presumed levels of peak formaldehyde exposure and average intensity of formaldehyde exposure*."[9]
Hauptmann *et al.* 2009	Duration of embalming practice and related formaldehyde exposures in the funeral home industry were associated with statistically significantly increased risk for mortality from myeloid leukemia. Mortality from myeloid leukemia increased statistically significantly with increasing number of years of embalming (P_{trend} = .020) and with increasing peak formaldehyde exposure (P_{trend} = .036).	A positive association was reported between embalming (ever worked) and myeloid leukemia; there was little evidence of an association with lymphohematopoietic cancers of lymphoid origin. The risk of myeloid leukemia increased with duration of employment as an embalmer and peak formaldehyde exposure, and was substantially elevated among those with the highest estimated cumulative exposure to formaldehyde.[10]

Thus, three of the four studies (Coggon *et al.* (2003), Pinkerton *et al.* (2004), Beane Freeman *et al.* (2009)) expressly state that they did not find a statistically significant association or exposure-response relationship between formaldehyde exposure and myeloid leukemia. **This consistent finding among three diverse studies which collectively involved more than 50,000 occupationally exposed workers calls into question the results of the fourth study (Hauptmann *et al.* 2009) and demonstrates that there is not "sufficient" evidence of an association with myeloid leukemia consistent with NTP listing criteria. This is particularly evident because the largest of these studies (Beane-Freeman *et al.* 2009) unequivocally does not show any significant association between formaldehyde exposure and myeloid leukemia in a cohort of more than 25,000 exposed workers.**

The following analysis of the Beane-Freeman *et al.* (2009) and Hauptmann *et al.* (2009) studies further supports the conclusion that there is insufficient evidence demonstrating an association between formaldehyde exposure and myeloid leukemia

[8] Beane Freeman *et al.* (2009) at p. 754.

[9] Expert Panel Report at p. 8.

[10] Id.

1. *Beane-Freeman et al. (2009)*

a. NCI Corrections Undercut Purported Leukemia Correlation

The most recent follow-up of the long-running NCI cohort study revealed that Hauptmann (2003, 2004) had missed 1,006 deaths among cohort members in the previous follow-up as already identified by Marsh and Youk (2004). This led to the 2009 online publication by NCI (Beane Freeman *et al.* 2009b) of corrected tables from the earlier 2003 and 2004 publications (Hauptmann *et al.* 2003; 2004). A key change in the original findings for leukemia (Hauptmann *et al.* 2003) was that NCI had missed proportionally more deaths among the low-exposed and unexposed subgroups that served as the baseline groups in the internal relative risk comparisons. This new finding is consistent with findings in the Marsh and Youk (2004) reanalysis, which showed that the exposure-response association for leukemia originally reported by Hauptmann *et al.* (2003) was due largely to statistically significant deficits in deaths among the low-exposed and unexposed subgroups.

The missed deaths change from significant to non-significant the trend reported for the exposed workers in the original 1994 follow-up, as shown in Table 2. The incorrect data was part of the decision-making basis for the International Agency for Research on Cancer (IARC) review in 2006.

Table 2. Correction of NCI 1994 Data and Additional Follow-up for Leukemia and Resulting Attenuated Exposure-Response Relationship

	NCI Study # Deaths RR (95% CI)			
Highest Peak (ppm)	1994 Follow-up Original[a,b]	1994 Follow-up Revised[c]	2004 Follow-up[d]	Marsh *et al.* (2004) re-analysis[e] SMR (95% CI)
Unexposed	4 0.78 (0.25-2.43)	4 0.52 (0.17-1.57)	7 0.59 (0.25-1.36)	0.38* (0.10-0.97)
>0-1.9 (base)	16 1.00 ----	23 1.00 ----	41 1.00 ----	0.50* (0.28-0.81)
2.0 – 3.9	20 2.04 (1.0-4.01)	20 1.36 (0.73-2.51)	27 0.98 (0.60-1.62)	1.04 (0.63-1.60)
>4.0	29 2.46 (1.31-4.62)	29 1.60 (0.90-2.82)	48 1.42 (0.92-2.18)	1.31 (0.88-1.89)

a: Used by IARC in 2004; b: P_{trend}=0.001 (all groups), P_{trend}=0.004 (exp. only); c: P_{trend}=0.021 (all groups), P_{trend}=0.094 (exp. only); d: P_{trend}=0.02 (all groups), P_{trend}=0.12 (exp. only); e: P<0.05

b. Observed Deaths from Leukemia in 3 of 4 Occupationally Exposed Cohorts are Exactly as Expected

When the Agency for Toxic Substances and Disease Registry (ATSDR) reviewed the carcinogenicity of PCBs (ATSDR 2000), it took a common sense approach by comparing the observed and expected mortality from all studies and using a statistical test to determine if there was a significant difference. When the same approach is applied to the three major human formaldehyde cohorts where this method can be used (Beane Freeman *et al.* (2009), Coggon *et al.* (2003), and Pinkerton *et al.* (2004)), a total of 152 cases were observed while 153.2 would be expected.[11] As shown in Table 3 the observed and expected leukemia mortality data from these studies of formaldehyde-exposed workers illustrate quite clearly that there is no excess.

[11] Hauptmann *et al.* 2009, an embalmer study, did not report observed and expected mortality.

Table 3: Comparison of Observed and Expected Leukemia Mortality in Formaldehyde-Exposed workers

	25,000	116	≈ 116
	14,000	12	13.2
	11,000	24	≈ 24
	50,000	152	153.2

This analysis of 50,000 formaldehyde industry workers demonstrates, as do more robust meta-analyses, that there is no marked excess of leukemia in these cohorts.

c. Meta-Analyses Show No Consistent or Collective Effect Regarding Leukemia

A number of meta-analyses have been conducted on the body of epidemiologic studies concerning formaldehyde and leukemia. Only the most recent, Bachand *et al.* (2010), includes the recent NCI study update. For cohort studies, Bachand *et al.* (2010) found that summary risk estimates (REs) ranged from 0.43 to 1.60 for leukemias, with all but one study having 95% confidence intervals, including 1.0. For two case-control studies the RE was 0.98 (95% CI: 0.70, 1.36) for Blair *et al.* (2001) and 1.40 (CI: 0.25, 7.91) for Partanen (1993). Meta-regression showed the overall leukemia RE was 1.05 (95% CI: 0.93, 1.20).

According to Bachand *et al.* (2010), earlier meta-analyses took inadequate notice of the potential for heterogeneity. Some may have had issues with selection bias. Of these, both Collins and Lineker (2004) and Bachand *et al.* (2010), stratify and analyze the data based on separate consideration of low-exposure and high-exposure industries while Zhang *et al.* (2008) does not. This may be important because it is notable that the high-exposure industries have, if anything, a lower collective indication of effect than the low-exposure industries. Collins and Lineker (2004) found a small but significant effect among embalmers (RR=1.6, CI:1.2,6.0) and a marginally significant effect among pathologists and anatomists (1.4, CI:1.0,1.9), both low-exposure professions, but no significant effect among higher-exposed industrial cohorts. Moreover, these medicine-associated job categories may be affected by diagnostic bias.

Zhang *et al.* (2008) found a significant effect across industries, however, they had a questionable means of selecting and combining studies. The authors used different measures of exposure, selecting only one from each study even if several were examined, resulting in their selection of peak exposure for some studies, average exposure for others, cumulative exposure for still others, and exposure duration for the balance. Moreover, if several categories or levels of exposure were examined, they took data from only the highest among them, and what constituted a "high" category also varied considerably among studies, depending on how each study established gradations of exposure. As a consequence, the comparisons across studies are very heterogeneous, and it is not clear whether a comparable question was being examined in each case, which can lead to unreliable results in a meta-analysis. The results in Zhang *et al.* (2008) should be interpreted with caution, especially in view of their lack of concordance with other meta-analyses.

2. Hauptmann et al. (2009)

Most of the studies on embalmers, pathologists, and anatomists report increased risk of leukemia. These findings have been attributed to either reporting bias, some exposure other than formaldehyde-related substances in the embalming, or to infectious agents (Harrington and Shannon (1975), Walrath and Fraumeni (1983, 1984), Stroup *et al.* (1986), Hayes *et al.* (1990)).

In the recent embalmer study of Hauptmann *et al.* (2009), formaldehyde exposure is never measured but rather is inferred from the number of embalmings.

Embalming fluids are complex mixtures including other chemicals along with formaldehyde. The mixture of chemicals in embalming fluids has changed over the years. Because the number of embalmings was one of the best predictors of risk of leukemia according to Hauptmann *et al.* (2009), it could be that another component of embalming fluids is related to the increased risk.

Due to the short time that this study has been available, it is possible that further analyses will reveal additional questions, but the following are some of the primary concerns with Hauptmann *et al.* (2009) (K. Mundt, personal communication).

- The study evaluates deaths occurring between 1960 and 1986 as reported on death certificates, but as late as 1992, NCI (SEER) did not report study results on cancer types being evaluated because SEER questioned the validity of these diagnoses as reported on death certificates.
- Important differences are apparent between myeloid (acute and chronic combined) leukemia cases and the control group and several time-dependent co-factors appear not to have been adequately considered or controlled in the analyses. This is a critical factor because myeloid leukemia is also a disease of aging.
- Standard statistical analyses were unreliable due to the fact that there was only one unexposed myeloid leukemia case.
- Myeloid leukemia cases and the controls had nearly identical mean estimated values for 8-hour time weighted average and peak formaldehyde exposure, which is inconsistent with the authors' interpretations.

B. *Animal Data*

According to the Expert Panel, “there is sufficient evidence for the carcinogenicity of formaldehyde from studies in experimental animals in two species, including multiple strains of rats at multiple sites using two routes of exposure” – inhalation and ingestion.

1. Exposure by Inhalation

With respect to the myeloid leukemia endpoint, while there are numerous animal inhalation studies with formaldehyde, these were essentially all conducted with the knowledge that nasal tumors were the only likely endpoint produced. Consequently, most studies simply did not look for lymphohematopoietic malignancies. This interpretation was consistent with abundant data demonstrating that distant site toxicity for formaldehyde was unlikely due to the fact that exogenous formaldehyde does not enter the body to change normal endogenous levels. Consequently, other than nasal tumors, distant site tumors for most studies were not investigated. Inhaled formaldehyde, even up to 15 ppm for 90 days, has no adverse effects on red or white blood cell counts or on the bone marrow. M. Andersen (personal communication). While there may or may not be species differences with respect to formaldehyde-induced hematotoxicity, the lack of any effects at 15 ppm following 90 days of exposure is striking.

2. *Exposure by Drinking Water*

The Expert Panel Report appears to have acted consistently with both the ATSDR and the U.S. Food and Drug Administration (FDA) in dismissing the findings from Soffritti *et al.* (1989).[12] NTP should act accordingly and not rely on Soffritti *et al.* (1989) in the draft substance profile or in the NTP listing decision. No other studies related to formaldehyde in drinking water, as cited in the Background Document, corroborate the conclusions of Soffritti *et al.* Based on all of the data, there is no credible evidence that formaldehyde causes tumors following ingestion.

In reviewing the results of Soffritti *et al.* (1989), ATSDR (1999) expressed skepticism: "*Another limitation to the strength of the evidence for formaldehyde-induced leukemia is the lack of a consistent dose-response relationship in the Soffritti et al. study . . . . The second part of the Soffritti et al. (1989) study found no statistically increased incidence of leukemia in groups of breeding pairs of rats or their offspring exposed for life to the higher dose level of 313 mg/kg/day. A further limitation is the absence of corroborating evidence for effects at sites distant from portals-of-entry in the other drinking water rat studies, and in inhalation-exposure animal studies.*"

The Cancer Assessment Committee of the FDA Center for Food Safety and Applied Nutrition also reviewed the study of Soffritti *et al.* (1989), concluding that the data reported were "*unreliable*" due to "*a lack of critical detail. . . questionable histopathological conclusions, and the use of unusual nomenclature to describe the tumors*." Consequently, the FDA "determined that there is no basis to conclude that formaldehyde is a carcinogen when ingested" (FDA, 1998).

As stated in a comprehensive review by Feron *et al.* (1991), "*Since, however, crucial information on procedures and histopathology of non-neoplastic changes is lacking, the adequacy of this study and the relevance of the data can hardly be judged, if at all*." Feron *et al.* (1990, 1991) noted that none of the contradictory findings from other oral dosing studies that were available when Soffritti *et al.* (1989) published their results were discussed. In addition, while Soffritti *et al.* present their historical control data for stomach, intestine, and gastrointestinal (GI) neoplasms in Sprague-Dawley rats, historical control data for lymphoblastic leukemia-lymphosarcoma are conspicuously absent. As described by Feron *et al.* (1990, 1991), historical untreated control data in Sprague-Dawley rats of the colony used, show that the incidence of leukemia varies widely, with reported spontaneous incidence rates similar to those reported by Soffritti *et al.* suggesting that treatment-related effects may have been unrelated to formaldehyde exposure.

Finally, Soffritti *et al.* (2002) again reported the results first published as Soffritti *et al.* (1989). This appeared to be the same study except that the reported incidence of leukemia was almost doubled in most treatment groups, that is, 45 versus 91 in males and 34 versus 60 in females. However, information on historical control incidences of leukemia was still lacking, and there was no explanation for the dramatic changes in the incidence of leukemia in the two reports.

The Expert Panel Report does not mention Tobe *et al.* (1989), another ingestion study in which formaldehyde was administered to rats in their drinking water at concentrations of 0, 0.02, 0.10 and 0.5 % for 24 months. While numerous tissues were examined for potential adverse effects, lymphohematopoietic malignancies were not specifically mentioned. However, red blood cells

[12] Expert Panel Report at 27-28.

(RBC), white blood cells (WBC), and hematocrit (Ht) were measured on each animal at necropsy. While there were some decreases in RBC and Ht these were not dose-related and, therefore, are not relevant. **Importantly, the fact that the white blood cell count was unaffected by the large doses used has implications for any proposed mode of action for formaldehyde-induced leukemia.**

In addition, Til *et al.* (1989) conducted another study in rats in which formaldehyde was administered in their drinking water at doses of 5, 25, 125 mg/kg for two years. Blood samples were collected from 10 rats/sex/dose group at 26 and 103 weeks and examined for RBC and WBC counts and Ht. While histopathology examinations did not include bone marrow, axillary lymph nodes were examined. **After two years of formaldehyde exposure via drinking water, there were no differences between dose groups in any hematological parameters, no dose-related lymphoma in axillary lymph nodes and no evidence of myeloid leukemia in blood cells.** This study did not provide any evidence of carcinogenicity from formaldehyde after oral administration.

C. *Other Data*

1. *Toxicokinetics*

The Expert Panel Report states: "*There is also indirect evidence that formaldehyde produced formaldehyde-DNA adducts in the blood of smokers (Wang et al. 2009) and DNA-protein crosslinks (DPCs) in the blood of formaldehyde-exposed hospital workers (Shaham et al. 2003, Shaham et al. 1996, Shaham et al. 1997).*"[13] It appears that this refers to the toxicokinetics of formaldehyde as it might pertain to the development of sinonasal cancer, NPC or myeloid leukemia. Consequently, it is reasonable to expect that there would be some evidence (with appropriate citations) demonstrating how formaldehyde-DNA adducts or DPCs are a marker for the development of any of the above three types of cancer. This would be of particular importance because it is well documented that both formaldehyde-DNA adducts and DPCs are normally found as a consequence of endogenous formaldehyde. Given their ubiquitous nature, their instability and rapid rates of repair, it is incumbent that these issues be more vigorously addressed.

a. Presence of Acetal (Methanediol)

The Expert Panel Report noted that: *"It is also well recognized that formaldehyde exists in equilibrium with methanediol and with S-hydroxymethylglutathione, both of which offer possible mechanisms for formaldehyde to enter the blood and be transported to other tissues*."[14] It is also well recognized that endogenous formaldehyde is present in the blood and therefore is transported to other tissues. The logic here applies only to the hypothetical myeloid leukemia endpoint because neither sinonasal cancer nor NPC require formaldehyde transport to distant sites. Although completely undocumented, it appears that this argument is intended to support the idea that exogenous formaldehyde raises endogenous blood concentrations, such that distant site tissues are exposed to levels that somehow overwhelm the well-documented prodigious metabolic capabilities of formaldehyde detoxification.

The Expert Panel Report language may be based on Zhang *et al.* (2010), which suggests that gaseous formaldehyde in the presence of water (from the blood) dissolves and is converted to

[13] Expert Panel Report at 26.
[14] *Id*.

its hydrated form, methanediol [$CH_2(OH)_2$] (also known as methylene glycol) and therefore could potentially reach the bone marrow in this form where free formaldehyde would then be released. As described by Zhang *et al.* (2010), *"methandiol…which can readily penetrate into tissues, may travel to the marrow through the blood where it is in equilibrium with reactive formaldehyde. The formaldehyde, once generated, can react with cellular macromolecules producing toxic injury."* **The cited basis for this statement is a publication by Fox *et al.* (1985) on the use of 4% formaldehyde solutions for tissue fixation. At this concentration, as intended, formaldehyde rapidly penetrates tissues to denature and cross-link proteins thereby also arresting enzymatic degradation of tissues.** Consequently, **it is unwarranted to hypothesize about the biological activity of formaldehyde based on extrapolating from tissue fixing concentrations of 4% (i.e., 40,000 ppm) to normal endogenous concentrations of 2-3 ppm which are approximately 10,000 times less.** In addition, because formaldehyde and methanediol are in equilibrium, the Expert Panel Report should also explain (with appropriate citations) **how and why this equilibrium changes at distant sites to release free formaldehyde and what it is about distant sites (as opposed to nearer the portal of entry) that causes this equilibrium to release free formaldehyde.** Such an explanation is needed particularly because the formaldehyde-methanediol equilibrium at 25^{o} C strongly favors methanediol and not formaldehyde (Matubayasi *et al.* (2007). Formaldehyde, as a non-hydrated aldehyde, predominates only in the air phase. Whether in the extracellular spaces or within cells, free formaldehyde will be present at extremely low concentrations since it first reacts reversibly with water to form an acetal (i.e., a more correct designation rather than methanediol) and then interacts with glutathione (GSH) to form a thioacetal. The equilibrium constant for acetal versus free formaldehyde is somewhere between 5,000 and 10,000. This issue, which lays at the heart of the biological plausibility of the Zhang *et al.* (2010) reported findings as a consequence of formaldehyde exposure, is of sufficient importance that NTP must avoid its uncritical acceptance. The findings described below by Lu *et al.* (in press) documenting that no exogenous FA-DNA adducts can be detected at any site distal to the nasal epithelium also must be addressed with respect to this issue.

b. Endogenous versus Exogenous Formaldehyde

The Expert Panel Report provides no references or data to support their conclusion that inhaled formaldehyde can increase endogenous levels of free formaldehyde. This conflicts with a large amount of empirical data on the chemistry and biochemistry of formaldehyde. This was well described in a critical review by Heck and Casanova (2004) in which the biological plausibility of formaldehyde-induced leukemia was assessed: "*An adult man (respiratory minute volume 12.0 ± 3.0 L/min (Malmberg et al. 1987)) would absorb 30 µg formaldehyde per minute if the formaldehyde concentration were 2 ppm. Assuming that 93% of the inhaled formaldehyde is eliminated by saturable metabolism in the respiratory tract as calculated for both rats and monkeys…the maximum amount of residual formaldehyde that would be available for distribution to other tissues would be 7%. (Note that this ignores other potentially important routes of elimination, such as nonsaturable metabolism in the respiratory tract, covalent binding to mucus proteins, and metabolism in the blood or other tissues.) If the residual formaldehyde were unmetabolized and distributed to total body water (41 L), its maximum concentration after 8 h would be less than 0.001 mM, which is well below the concentration of endogenous formaldehyde in human blood (≈ 0.1 mM). Of course, metabolism in the blood and tissues would greatly reduce the actual concentration of residual formaldehyde in total body water. Therefore, inhaled formaldehyde would not be expected to increase the formaldehyde concentration in the blood in accordance with the empirical results.*" The empirical results referenced in the last sentence refer to studies in rats, monkeys and humans demonstrating that inhaled formaldehyde up to almost 15 ppm (rats) and 6 ppm in monkeys for 4 weeks does not change endogenous concentrations (Casanova *et al*. 1988, Heck *et al*. 1985)

Of direct relevance to the above, the issue of distant site toxicity was recently investigated in an elegant study by Lu *et al.* (in press), in which rats were exposed via inhalation to ^{13}C-formaldehyde at 10 ppm for 1 and 5 days. This protocol used mass spectrometry to determine whether formaldehyde-DNA adducts in numerous tissues (i.e., nasal epithelium, liver, spleen, lung, bone marrow and blood) were derived from exogenous (i.e., ^{13}C-formaldehyde) or endogenous (i.e., ^{12}C-formaldehyde) formaldehyde. **While formaldehyde-DNA adducts from both endogenous and exogenous formaldehyde were readily detected in nasal epithelium after 1 or 5 days, no formaldehyde-DNA adducts from exogenous formaldehyde were detected in any site distal to the nose, including the bone marrow and white blood cells. The demonstration that exogenous formaldehyde does not get past the nasal epithelium confirms prior work and calls into question whether the findings reported by Zhang *et al.* (2010) involving changes** in blood counts and in two chromosomes can be attributed to formaldehyde. Simply stated, the results of Lu *et al.* (in press) and Zhang *et al.* (2010) cannot both be correct. Given the central issue concerning exogenous formaldehyde reaching distal sites, NTP should not rely on the results of Zhang *et al.* (2010) alone.

The Expert Panel "*recognized that the endogenous levels of formaldehyde-methanediol in human blood are high (about 0.1 mM; Heck and Casanova 2004) and that this represents a significant challenge for low-dose extrapolations*." The naturally occurring endogenous levels of formaldehyde-methanediol in human blood only represent a significant challenge for low-dose extrapolations if such methods fail to take this into account. It is because of these endogenous levels of formaldehyde that breath concentrations of ≈ 1-2 ppb are continuously exhaled. The existence of formaldehyde as a normal human metabolite and its wide-ranging presence in plants and animals, calls into question any finding of risk from low-level human exposure. The significant challenge is to scientifically justify low-dose extrapolations from any risk assessment methodology that projects cancer risk at exposures that are 1 to 2 orders of magnitude below normal human breath levels of exhaled formaldehyde as well as in ambient air.

c. Chromosomal Damage

As noted in the Expert Panel Report: "*the recent study of Zhang et al. (in press) showed evidence of aneuploidy in human chromosomes 7 and 8 in myeloid progenitor cells from formaldehyde-exposed workers*."[15] Since this study played a pivotal role in the conclusions reached by IARC concerning an association between formaldehyde exposure and leukemia, it is surprising that the Panel did not address its strengths and weaknesses. We question whether the Panel, in apparently accepting at face value the findings reported by Zhang *et al*. (2010), considered that:

- Chromosomes 7 and 8 are minimally relevant to leukemia and their count number in peripheral blood lymphocytes is not known to have any predictive value.
- Even if chromosomes 7 and 8 were relevant, the methods used to evaluate them are not credible. The study would have to be repeated if it were to be acceptable for publication in the hematology or pathology scientific literature.

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[15] *Id.*

- There is no existing accepted diagnostic test in clinical medicine, hematology or hematopathology that can establish the presence of leukemia, or increased risk of developing leukemia, by detection of monosomy 7 or trisomy 8 in cultured myeloid progenitor cells from peripheral blood.
- Only Chromosomes 7 and 8 were examined while ignoring all other chromosomes, translocations and any of the common genetic lesions associated with leukemia?
- Chromosomes 7 and 8 are not usually involved in leukemia. For example in 122 AML patients in China, none had monosomy 7 and only 4 had trisomy 8.[16]

The Expert Panel Report notes that "*In light of the propensity of formaldehyde to damage chromosomes in mammalian cells, it is also important to emphasize that chromosome aberrations are the only validated biomarkers of human cancer (Bonassi et al. 2008).*" However, the study by Bonassi *et al.* (2008), which is based on genetic screening in 22,358 ***cancer-free*** individuals with follow-up for an average of 10 years, is cited as support for the hypothesis that chromosomal aberrations are the only validated biomarker of human cancer. It is important to note, that the only cancer site significantly associated with the frequency of chromosomal aberrations reported by Bonassi *et al.* (2008) is stomach cancer (Bonassi *et al.* 2008), which, as acknowledged by the authors, could well be a consequence of multiple comparisons. Furthermore, there is no evidence that the frequencies of chromosomal effects reported result from chemical exposure (there were no specific exposure measurements in any of the groups studied) and not from intrinsic genomic instability and/or repair capacity.

Of particular relevance to the issue of formaldehyde-induced chromosomal aberrations (CA) as risk factors for myeloid leukemia, Bonassi *et al.* (2008) reported no significant association between chromosomal aberrations and increased relative risk of malignancies of the lymphohematopoietic system. As noted by Bonassi *et al.* (2008): "*Another open issue is the role of exposures to genotoxic and carcinogenic agents experienced by study subjects at the time of CA testing. A nested case-control study carried out within the Nordic–Italian databases to specifically test the presence of interaction of CA frequency with major occupational exposure to carcinogens and with cigarette smoking did not find any difference in the risks.*" Consequently, studies such as this provide no support for the assumption that chromosomal aberrations measured in cultured lymphocytes are an indication of increased cancer risk. This applies broadly to cancer risk in general and even more so to specific types of cancer such as myeloid leukemia.

d. Hematotoxic Effects

The Expert Panel Report does not address the key issue of whether exposure to formaldehyde had any hematotoxic effects. Hematotoxicity (*i.e.*, statistically significant decreases in red and white blood cell counts) was reported by Zhang *et al.* (2010), and was superficially reviewed by Tang *et al.* (2009), a study cited in the Expert Report and extensively summarized in the NTP formaldehyde Background Document.

This is a key issue because hematotoxicity, *e.g.*, pancytopenia, which is an indicator of myelotoxicity, has been associated with all known human leukemogenic chemicals and is a necessary precursor for chemical leukemogenesis. Tang *et al.* (2009) briefly review a number of studies that purport to demonstrate that exposure to formaldehyde is a cause of

[16] Zheng, et al. Cytometry Part B: Clinical Cytometry, 74B, pages 25-29, 2007.

hematotoxicity. As summarized in Table 9 of Tang *et al.* (2009) from the various studies cited, while some formaldehyde levels are listed, it is not possible to know what additional exposure conditions were present in order to evaluate whether the reported results on white blood counts, platelet counts or hemoglobin levels were due to formaldehyde or another exposure. For example, Tang *et al.* (2009) cite a study by Kuo *et al.* (1997) in support of adverse hematological effects. This study was conducted on 50 hemodialysis nurses and controls from four hospitals in Taiwan and concluded that the white blood cell counts were significantly lower in the exposed group compared to controls. However, this study is not credible because the formaldehyde analytical data are suspect and the overall formaldehyde levels were implausibly low (e.g., mean personal sampling concentrations of 0.015 ppm, 0.017 ppm, 0.033 ppm and 0.054 ppm) in the 4 hospitals. These levels are similar to the exposure levels of controls in Zhang *et al.* (2010).

The majority of more credible data show essentially no reported adverse hematological effects following exposure of either humans or animals to formaldehyde. This has substantial implications with respect to any hypothesized mechanism for formaldehyde-induced myeloid leukemia. No matter how one might speculate that this occurs (e.g., formaldehyde-induced myelotoxicity or formaldehyde-induced mutations to stem cells with subsequent transport to the bone marrow), all would require pancytopenia as an early indicator of potential disease.

- While an accidental ingestion of a large quantity of formaldehyde was reported to cause an intravascular coagulopathy (Burkhart *et al.* 1990), several reports of human ingestion of lower doses have not shown any effects on the blood or blood-forming organs (Eells *et al.* 1981, Freestone and Bentley 1989, Koppel *et al.* 1990).
- In animal studies, neither inhalation exposure (Appelman *et al.* 1988, Kamata *et al.* 1997, Kerns *et al.* 1983, Woustersen *et al.* 1987) nor oral exposure (Johannsen *et al.* 1986, Til *et al.* 1989, Tobe *et al.* 1989) to high doses of formaldehyde has produced any evidence of adverse hematological effects.
- A single study in rats exposed to massive oral doses of formaldehyde (e.g., 80 mg/kg for 4 weeks) reported minor increases in erythrocyte count and hemoglobin values (Vargova *et al.* 1993).
- As noted in ATSDR (1999), the lack of hematopoietic toxicity in these studies is *"likely related to rapid metabolism prior to the formaldehyde reaching the blood and blood-forming components (bone marrow)."*

Many of the above-cited studies are included in the Background Document and demonstrate that formaldehyde is unlikely to cause adverse hematological effects. Moreover, in a recently completed 90-day inhalation study with formaldehyde at exposure concentrations of 0, 0.7, 2, 6, 10 and 15 ppm there were no effects on red or white blood cell counts at any exposure level nor were there any effects on the bone marrow (M. Andersen, personal communication). These data indicating a lack of either formaldehyde-induced hematotoxicity or myelotoxicity and call into question the source of the reported changes as described by Zhang *et al.* (2010). While there can be debate on the sensitivity of rodents versus humans, the striking lack of effects on blood or bone marrow at formaldehyde exposures up to 15 ppm for 13 weeks questions whether the reported exposure in the Zhang *et al.* (2010) (i.e., median exposure of 1.28 ppm) was capable of causing adverse hematotoxic effects.

D. *Final Myeloid Leukemia Observations*

The human, animal and other data do not provide evidence adequate to support a listing determination by NTP based on an association between formaldehyde exposure and myeloid leukemia; instead the data are highly equivocal. The evidence from Hauptmann *et al.* (2003) hinges substantially on the statistically significant mortality deficits in the low- and non-exposed internal comparison groups and, as shown by Marsh *et al.* (2004), the finding is no longer apparent when using external comparisons. Even with these deficits, **the follow-up by Beane-Freeman *et al.* (2009) unequivocally shows no significant association between myeloid leukemia and any exposure metric. The revelation by Beane-Freeman *et al.* (2009) of the 1000+ missing deaths in the Hauptmann *et al.* (2003) study, and the unequivocal effect of this finding on the leukemia findings in this study, is further evidence of the weakness of the association between formaldehyde exposure and leukemia in general and myeloid leukemia in particular. Additionally, neither of the other two large cohort studies by Coggon *et al.* (2003) or Pinkerton *et al.* (2004) show statistically significant increases in myeloid leukemia.** The only study suggesting an association is the embalmers study by Hauptmann *et al.* (2009) and the numerous limitations of this study are discussed above. Moreover, two of the three meta-analyses of the epidemiological data (i.e., Collins and Lineker 2004 and Bachand *et al.* (2010), consistent with the Beane Freeman *et al.* (2009), Coggon *et al.* (2003), and Pinkerton *et al.* (2004) studies, show no association between exposure to formaldehyde and leukemia, and none of these are capable of showing separately any data for myeloid leukemia.

The study by Zhang *et al.* (2010) has generated considerable controversy because it is at odds with a substantial body of prior data and itself concedes the need for further research and validation. While all agree that there is a critical need for the results of this study to be replicated, the numerous shortcomings are simply too substantial for this study to be afforded much weight until it can be replicated. As explained above, the four most informative studies as identified by the Expert Panel Report clearly do not support the conclusion that formaldehyde is etiologically associated with myeloid leukemia based on the NTP evaluation criteria.

While the Expert Panel Report, Zhang *et al.* (2010), and the most recent report from IARC purport to offer an explanation for how exogenous inhaled formaldehyde can enter the blood to raise endogenous levels thereby initiating a sequence of events leading to the development of myeloid leukemia, there is a lack of credible data offered in support of this speculation. Instead, we are left with unfounded speculations that are not in agreement with: (1) empirical, peer-reviewed, published chemical, biochemical and biological data which convincingly demonstrate that inhaled formaldehyde does not change endogenous blood levels and (2) newer data showing that while endogenous formaldehyde-DNA adducts are found in all tissues examined, exogenously formed ^{13}C-formaldehyde-DNA adducts are found only in nasal epithelial tissues and not at any distant sites, including the blood and bone marrow. Lu *et al.* (in press).

III. Nasopharyngeal Cancer

A. *Human Data*

The Panel correctly states that the "*only cohort study that is individually informative*" for evaluating the potential carcinogenicity of formaldehyde and NPC is the NCI cohort of workers in formaldehyde industries by Hauptmann *et al.* (2004). The study conducted by NCI evaluated a group of more than 25,000 industrial workers at 10 U.S. industrial plants where formaldehyde was either produced or used in the production of other products. There was a total of 9 deaths from NPC, **with *5 of the cases coming from only one plant (Plant #1) and the remaining four cases randomly occurring in the other nine plants, an atypical pattern if formaldehyde were actually the cause of NPC.*** For NPC in the total cohort, the SMR was 2.10 (95% CI 1.05 -4.21) with significant relative risks (RR) associated with peak (P_{trend} <0.001) and cumulative P_{trend} =0.025) exposures, respectively.

While these RR appear substantial, an independent analysis of Plant #1 by Marsh *et al.* (2002) casts doubt on a formaldehyde-NPC association by showing that:

- the workers comprising 4 of the NPC cases had worked < 1 year (See Table 2, Marsh *et al.* 2002)
- 5 had worked < 5 years, and
- the average intensity of exposure was low, with a median concentration of 0.14 ppm.

Because of the short duration of employment and low exposure level, a causal association between formaldehyde exposure and NPC becomes highly speculative. Additional detailed analysis of the NCI data by Marsh *et al.* (2007) has provided further evidence that the NPC reported in this cohort may not be related etiologically to formaldehyde exposure at all. That analysis involved a careful investigation of the previous employment history of the individuals at the one plant who died from NPC. **Five of the six NPC cases at this plant had previously worked in occupations involving substantial exposures to potential risk factors for upper respiratory system cancers, including sulfuric acid mists and metal dusts.** According to the authors, *"The results of our nested case–control study suggest that the large nasopharyngeal cancer mortality excess in plant #1 may not be due to formaldehyde exposure, but rather reflects the influence of external employment in the ferrous and nonferrous metal industries of the local area that entailed possible exposures to several suspected risk factors for upper respiratory system cancer (e.g., sulfuric acid mists, mineral acid, metal dusts and heat). Our findings may also help to explain why the associations with formaldehyde and nasopharyngeal cancer reported in the 1994 update of the 10-plant NCI formaldehyde cohort study were unique to plant #1."* If the five cases of NPC with previous confounding exposures were excluded from the Hauptmann *et al.* (2004) study, it is reasonable to assume that there would be no significant association between formaldehyde exposure and NPC. This was confirmed in the meta-analysis conducted by Bachand *et al.* (2010).

The Expert Panel Report refers to a previous reanalysis by Marsh *et al.* (2005) and concludes that "*The comparatively high number of cases in Plant 1 may be due to potential confounding from an **unidentified agent***".[17] There is a more relevant analysis of Plant 1 by Marsh *et al.* (2002) as summarized above and the biological plausibility of previous exposures to known risk factors for NPC (i.e., Marsh *et al.* 2007). The analyses by Marsh *et al.* (2002, 2007) should be addressed. In particular, following identification of the strong likelihood of previous exposures to known risk factors for NPC, Marsh *et al.* (2007) provided extensive documentation of the association between these exposures and NPC as a more likely explanation then formaldehyde exposure.[18]

[17] Expert Panel Report at 21 (emphasis added).

[18] As summarized by Marsh *et al.* (2007), "For example, in 1992 IARC classified occupational exposures to strong inorganic-acid mists containing sulfuric acid as carcinogenic to humans (Group 1) based on sufficient epidemiological evidence. In particular, **mineral acid and sulfuric acid mists and vapors**

B. *Animal Data*

Advances in understanding the MOA by which chemicals induce tumors in rodents can play a key role in determining the relevance to humans of animal data. This is particularly the case with respect to formaldehyde-induced nasal tumors in rodents where MOA data suggest that the epidemiological data are not indicative of a causal association.

1. Exposure by Inhalation

Rat studies showing nasal cancer following chronic exposure to sufficient concentrations of formaldehyde appear to provide support for the biological plausibility of the epidemiological findings of NPC. Rat nasal tumors have been demonstrated in at least six chronic inhalation studies with formaldehyde (e.g., Kerns *et al.* 1983; Tobe *et al.* 1989; Sellakumar *et al.* 1985; Feron *et al.* 1989). In what is generally considered to be the definitive study of formaldehyde-induced nasal cancer, rats were exposed by inhalation to formaldehyde at concentrations of 0, 0.7, 2, 6, 10 and 15 ppm for two years (Monticello *et al.* 1990). **Nasal tumors occurred only at formaldehyde concentrations of > 6 ppm. The concentrations which produce tumors are far in excess of tolerable irritant levels for humans** and are sufficient to cause severe cytotoxicity of the rat nasal epithelium, with subsequent regenerative proliferation observed. Moreover, in contrast to humans, rats are obligatory nose breathers. Exposure at 6 ppm and above for a sufficient duration produces severely damaged cells that can develop into nasal tumors.

The key finding in rat studies of formaldehyde is that the sequence of events leading to nasal tumor formation occurs only at formaldehyde doses sufficient to produce cytotoxicity and regenerative proliferation. This general mode of action has been critically evaluated by McGregor *et al.* (2006) following EPA's Guidelines for Cancer Risk Assessment (EPA 2005) and in conjunction with the methods and approaches established by the International Life Sciences/Risk Sciences Institute (ILSI-RSI) and International Program on Chemical Safety (IPCS) (i.e., Cohen *et al.* 2003, 2004; Meek *et al.* 2003, Boobis *et al.* 2006). This overall methodology provides a decision-logic-based approach to determining the relevance to humans of laboratory animal study results. Using this methodology, McGregor *et al.* (2006) determined that of all the MOA elements (i.e., cytotoxicity, cell proliferation, and DNA effects) for formaldehyde-induced tumors, nasal are highly non-linear and do not occur unless a particular threshold dose (6 ppm) has been exceeded. The authors concluded that: "*From a weight-of-evidence point of view, the hypothesized mode of action for formaldehyde-induced nasal tumors satisfies several criteria, including consistency, concordance of dose-response relationships across all key events, and biological plausibility and coherence of the database. Given the extensive experimental data that addresses and is consistent with the proposed mode of action of formaldehyde in the induction of tumors in the nasal cavity, a high degree of confidence may be ascribed to it.*"

A recent *in vivo* study conducted subsequent to the above analysis adds further weight to the conclusions of McGregor *et al.* (2006). In this study, F-344 rats were exposed to formaldehyde

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have been associated with increased risks of upper respiratory tract cancers, including nasopharynx (NPC) (Ho *et al.* 1999; Li *et al.* 2006), larynx (Soskolne *et al.* 1984, 1992; Forastiere *et al.* 1987; IARC, 1992; Coggon *et al.* 1996; Steenland, 1997; Steenland *et al.* 1998; Sathiakumar *et al.* 1997)….Exposures to metal dusts have been linked to increased risks for NPC (Armstrong *et al.* 2000) and laryngeal cancer (Shangina *et al.* 2006), and industrial heat exposure has been linked to NPC (Armstrong *et al.* 2000)."

at concentrations of 0, 0.7, 2, 6, 10 or 15 ppm for 13 weeks with nasal epithelial tissues examined for the presence of one of the *p53* mutations that had been detected in the squamous cell carcinomas induced by chronic formaldehyde exposure in a two-year bioassay. In addition, because regenerative cell proliferation is considered a key event in formaldehyde-induced carcinogenesis (McGregor *et al.* 2006), nasal mucosal cell proliferation was monitored by bromodeoxyuridine (BrdU) incorporation. While there was a low spontaneous background level of *p53* mutation, this level was not increased by formaldehyde exposure, even at tumorigenic doses. However, when measured by BrdU labeling, the percentage of proliferating cells increased with formaldehyde dose and was significantly increased at 10 and 15 ppm compared to controls. These data, show no increase in *p53* mutation but significant changes in regenerative cell proliferation following 13 weeks of formaldehyde exposure at tumorigenic doses, and, therefore, suggest that *p53* mutation is a late event not involved in the carcinogenic MOA in formaldehyde-induced carcinogenesis and occurs only after other key events (e.g., DNA-protein crosslinks, cytotoxicity, cell proliferation) have occurred (Meng *et al.* 2009)

The detailed understanding of the likely MOA for formaldehyde-induced nasal tumors in rats, with a clear threshold necessary to produce this effect, sheds light on the biological plausibility that either NPC or sinonasal cancer might be caused by occupational exposure to formaldehyde. With the necessity for continuous exposure to formaldehyde at concentrations >6 ppm, it is implausible that anyone could or would tolerate exposures at this level for the time required to trigger the sequence of events that could lead to such tumors. Consequently, the animal and MOA data are supportive of the various critiques and meta-analyses of the epidemiological data which suggest that formaldehyde does not play an etiological role in the development of NPC. This available data merits consideration by NTP.

C. *Other Data*

1. *Genotoxicity Data*

The Expert Panel Report notes that "*It is clear from studies with in vitro model systems involving bacterial, mammalian, and human cells that formaldehyde is genotoxic*."[19] However, while recognizing that this Report must necessarily condense a great deal of the available data (as opposed to the Background Document), it was inappropriate to cite a single 10-year-old study by Merk and Speit (1998). The Panel Report apparently relied upon this study to conclude that formaldehyde acts as via a clastogenic mechanism (i.e., by CA) in mammalian cells with the assertion that formaldehyde would exert similar effects to produce tumors in sinonasal-pharyngeal cells. Speit *et al.* have published numerous additional studies (that are cited in the Background Document) that clearly challenge this notion. With respect to myeloid leukemia, while there might be evidence of formaldehyde-induced genotoxicity in peripheral lymphocytes, there is no basis for assuming that this is in any way linked to or associated with development of this disease. *See, e.g.,* Schmid and Speit (2007).

The Expert Panel Report notes that: "*In addition, Costa et al. (2008) reported DNA damage (comet assay) in lymphocytes from formaldehyde-exposed workers; this finding is supported by the review of Chinese studies summarized by Tang et al. (2009).*"[20] Costa *et al.* (2008) found an **increase** in DNA migration, while formaldehyde in inducing DPC very efficiently causes a **decrease** in DNA migration. This indicates that the results of this study are not related to formaldehyde exposure.

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[19] Expert Panel Report at 26.

[20] Expert Panel Report at 27.

2. Mechanistic Data

The Expert Panel Report states that there *"are two proposed mechanisms of formaldehyde carcinogenicity, namely, a cytotoxicity-induced-cell-proliferation (CICP) mechanism and a genotoxic mechanism. Regarding tumors in the sinonasal-pharyngeal regions (point of contact), evidence supports both these mechanisms in animal studies*."[21] While it is correct that cytotoxicity-induced-cell-proliferation plays a key role in nasal tumors, there is no evidence (and none is cited) that genotoxicity is initially involved early in the tumorigenic process. Instead, as noted by McGregor *et al.* (2006), *"Prolonged exposure to formaldehyde above a critical concentration induces sustained cytotoxicity and cell proliferation. As a result of genetic changes within this proliferating cell population, neoplasia emerges. The genetic changes are postulated to be secondary to the cytotoxicity, metaplasia, and hyperplasia that are clearly induced by formaldehyde."* As discussed above, the recent study by Mang *et al*. (2009) is further confirmation that genetic changes do not play a role in the development of nasal tumors.

Formaldehyde-induced nasal tumors in rats are indisputably a highly nonlinear threshold event with exposures > 6 ppm required to produce tumors. **Because of the highly irritating properties of formaldehyde, it is unlikely that anyone could or would spend prolonged periods of time exposed at high concentrations where nasopharyngeal carcinogenic effect has been observed**.

As further documentation of the nonlinear nature of formaldehyde-induced nasal tumors, Andersen *et al.* (2008) conducted a study in which rats were exposed to the same doses (0, 0.7, 2 and 6 ppm) of formaldehyde that had been used to characterize the nasal tumorigenicity threshold in the definitive chronic bioassay (Monticello *et al.* 1996), 5 days/week for three weeks, with interim sacrifices. Nasal epithelium was taken from the same locations at which tumors had developed in rats exposed to 10 and 15 ppm in the chronic bioassay and evaluated by histopathology and microarray analysis. No genes were significantly altered at 0.7 ppm at any time point, indicating a clear threshold for formaldehyde-induced effects. On day 5, a few genes were significantly changed at 2 ppm and many more genes were changed at 6 ppm. Most importantly, no genes were significantly changed at 2 ppm by day 15 of this study. These data show that even at 2 ppm, nasal cells initially show some minor effects, but after a few days the tissues rapidly adapt to formaldehyde at this concentration and return to a pattern of gene expression identical to 0 and 0.7 ppm. This study provides empirical support at the genomic level for the conclusion that formaldehyde exposure at concentrations of 2 ppm and less are incapable of causing tissue damage that could lead to tumor formation.

A 90-day inhalation toxicogenomic study has recently been completed in F344 rats using the same formaldehyde doses used in the Monticello *et al.* (1996) chronic bioassay (0, 0.7, 2.0, 6.0, 10, 15 ppm)(M. Andersen, personal communication). Tissues were collected for genomic analysis following 5, 28, and 90 days of exposure. Preliminary results show that no genes were altered at the 0.7 and 2 ppm doses at any time point including up to 90 days, while a dramatic increase in altered genes was observed at the 6 ppm and higher concentrations. While not yet published, these results will shed further light on the specific genes affected by formaldehyde, their likely roles in chronic disease processes, and their dose-response relationships.

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[21] Expert Panel Report at 28.

IV. Sinonasal Cancer

The category "sinonasal cancer" used in most studies combines nasal cavity cancers and nasal sinus cancers into a single category. The experimental studies on rats and mice both show excesses of nasal cavity cancer at high exposure levels with no mention of the nasal sinuses. Formaldehyde, however, does not penetrate into the nasal sinuses in rats and non-human primates where most of the human "sinonasal cancers" occur (Heck *et al.*1989). Ninety percent of the "sinonasal cancers" in humans are of the sinuses and not of the nasal cavity (NCHS, 1985). It would be more informative if studies examined only nasal cavity cancer, but few studies have done this. Virtually, all of the studies referenced in the Expert Panel Report use the category "sinonasal cancers," which may bias the relative risk estimates. NTP should consider this in preparing the draft substance profile and its listing determination.

The epidemiological evidence for formaldehyde-related carcinogenicity for sinonasal cancer is very limited and should not be used as a basis for reclassifying formaldehyde as a known human carcinogen. Thus, without explanation, the Expert Panel Report omitted three important and informative meta-analyses: Partanen (1993), Collins, *et al.* (1997), and the recent meta-analysis by Bosetti *et al.* (2008). These meta-analyses demonstrate that there was no increased risk of sinonasal cancer in the cohort studies (Bosetti *et al.* 2008; Collins *et al.* 1997). And while the case-control studies in the meta-analyses demonstrate an increased risk for this cancer (Collins *et al.* 1997; Partanen, 1993), many of these case-control studies also report exposure to wood dust as a potential confounding exposure. The Collins *et al.* (1997) meta-analysis stratified the high wood dust studies from the low wood dust studies, and found that the increased sinonasal cancer risk only occurred among the high wood dust workers.

Many of the same factors discussed above with regard to animal and other data that are relevant to NPC also are applicable to sinonasal cancers. In Hauptmann *et al.* (2004), only three cases (i.e., nose and nasal cavity) were observed in the cohort, and this was not statistically significant (SMR=1.19, 95% CI 0.38 – 3.68) (Table 2, Hauptmann *et al.* 2004).

Based on the same data, IARC concluded that there was “limited” evidence of sinonasal cancer in humans, while it appears that the Expert Panel, by a 9-0 vote, found the evidence to be sufficient for a finding that formaldehyde is a “known” human carcinogen with respect to sinonasal cancer. The Expert Panel did not align the data with the three endpoints that it examined.

V. Expert Panel Report

A. *Listing Criteria*

For a chemical to be listed as *Known To Be A Human Carcinogen*, it must meet the criterion that:

> There is ***sufficient evidence*** of carcinogenicity from studies in humans, which indicates a causal relationship between exposure to the agent, substance, or mixture, and human cancer.[22]

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[22] *See* Listing Criteria at http://ntp.niehs.nih.gov/index.cfm?objectid=03C9CE38-E5CD-EE56-D21B94351DBC8FC3.

NTP classifies substances as *Reasonably Anticipated To Be Human Carcinogen* based on the following criteria:

> There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded,
>
> or
>
> there is sufficient evidence of carcinogenicity from studies in experimental animals, which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site, or type of tumor, or age at onset,
>
> or
>
> there is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance, or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either known to be a human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.[23]

B. *Expert Panel Recommendations and Evaluation*

The Expert Panel: (i) suggests revisions to the draft Background Document, (ii) makes listing recommendations, and (iii) provides the rationale for its listing recommendation in its Report. The Panel's rationale should reference sufficient data and contain adequate analysis to justify NTP's reliance on the Panel's listing recommendations. The Expert Panel stated in its overall evaluation of formaldehyde: "*The panel identified epidemiological studies of workers exposed to formaldehyde that indicated a causal relationship between exposure to formaldehyde and cancer in humans. These studies, in a variety of unrelated occupations, found evidence of significant excess of three types of cancer with a positive dose-response relationship: nasopharyngeal carcinoma (NPC), sinonasal adenocarcinoma and myeloid leukemia. Chance, bias, and confounding are unlikely to explain the observed excess in these cancers.*"[24]

FCI disagrees with this evaluation. Given the discrepancies between the available scientific data and the description of the data in the Panel's Report, the Panel did not give due consideration to the information before it. Examples of the Report's flaws include:

1. References to leukemia and myeloid leukemia are inappropriately used interchangeably, as if they are one and the same disease, and by stating 'leukemia' as if there is a causal relationship between formaldehyde and every

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[23] *Id.*

[24] Expert Panel Report at p. 2.

form of leukemia.[25] The Expert Panel Report is quite clear that only myeloid leukemia was a focus of concern.

2. The Panel states that it found evidence of significant excess of three types of cancer with a positive dose-response relationship. Yet throughout the body of the report, the Panel reverts to the simple metric of “excess” and not “statistically significant excess.” Furthermore, there is scant evidence of a positive dose-response (or exposure-response) relationship, particularly for myeloid leukemia.
3. The Panel summarizes its findings by stating that it "focused on three sites for which the evidence was strongest and most ***consistent*** . . . ."[26] As discussed above, the studies relied on by the Panel, particularly for myeloid leukemia, are not consistent but rather highly inconsistent with respect to a causal association with myeloid leukemia.

The science does not support many of the Report's conclusions. This confusion likely arose from the Panel taking a single vote on the scientific evidence for all three endpoints, as opposed to assessing each endpoint individually. For these reasons, we recommend that NTP reconvene the Expert Panel to not only assess the data based on the three, separate toxicological end points, but also, using a weight-of-evidence approach, to classify each endpoint separately as to the potential association with formaldehyde exposure, and to re-poll the Panel as to its view on whether the data are sufficient, limited or inconclusive with respect to each end point.

VI. Conclusion

Based on a review of the science and the application of NTP's criteria, regardless of whether one applies a weight-of-evidence or strength-of-evidence approach:

- The human, animal and other data for myeloid leukemia do not provide sufficient evidence that supports listing formaldehyde as either *known to be a human carcinogen* or *reasonably anticipated known to be a human carcinogen.*
- The human, animal and other data for nasopharyngeal cancer (NPC) do not provide sufficient evidence that supports listing formaldehyde as a *known to be a human carcinogen*, but may be interpreted to support a listing as *reasonably anticipated to be a human carcinogen*,
- The human, animal and other data for sinonasal cancer do not provide sufficient evidence that supports listing formaldehyde as either *known to be a human carcinogen* or *reasonably anticipated known to be a human carcinogen.*

The evidence does not support the Panel's conclusions that any of the three cancer types identified should be considered a known human carcinogen.

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[25] According to a chronic/acute and lympocyte/myeloid criteria, there are five main types of leukemia: (1) Chronic Lymphocytic Leukemia; (2) Chronic Myeloid Leukemia; (3) Acute Lymphocytic Leukemia; (4) Acute Myeloid Leukemia and (5) Hairy Cell Leukemia.

[26] Expert Panel Report, p. 12 (emphasis added).

If you have any questions or seek additional information from FCI and its science consultants, please do not hesitate to contact me at 703-875-0710 or bnatz@formaldehyde.org.

Sincerely,

signature redaction [redacted]

Betsy Natz
Executive Director
Formaldehyde Council, Inc.

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